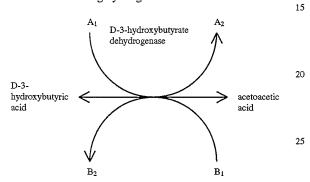
phosphate or its analog (thio-NADP compound) and thionicotinamide adenine dinucleotide or its analog (thio-NAD compound), and

(ii) a second coenzyme selected from the group consisting of nicotinamide adenine dinucleotide 5 phosphate or its analog (NADP compound) and nicotinamide adenine dinucleotide or its analog (NAD compound),

and catalyzes the reversible reaction producing acetoacetic acid from D-3-hydroxybutyric acid as a <sup>10</sup> substrate;

- (2) A<sub>1</sub>; and
- (3) B<sub>1</sub>; said components (1), (2) and (3) participating in the following cycling reaction:



wherein  $A_1$  is a thio-NADP compound, a thio-NAD compound, an NADP compound or an NAD compound;  $A_2$  is a reduced form of  $A_1$ ;  $B_1$  is a reduced NADP compound or a reduced NAD compound when  $A_1$  is a thio-NADP compound or a thio-NAD compound, or a reduced thio-NADP compound or a reduced thio-NADP compound or a reduced thio-NADP compound or an NADP compound or an NADP compound; and  $B_2$  is an oxidized form of  $B_1$ , wherein the reaction of said biological sample with said reagent is conducted under conditions adapted for the enzymatically catalytic and cycling reaction, thereby effecting the cycling reaction; and

measuring and correlating a change in the amount of  $A_2$  or  $B_1$  to the quantity of said D-3-hydroxybutyric acid, wherein each of  $A_1$  and  $B_1$  is used in a concentration of 0.02 to 100 mM or more, and the D-3-hydroxybutyrate dehydrogenase is used in a concentration of 5 to 1,000 U/ml or more.

\* \* \* \* \*